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Optimization of an anti-HER2 nanobody expression using the Taguchi method

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ABSTRACT

Despite being widely used in immunotherapy of cancer, whole antibodies are limited by several disadvantages. This has led to the advent of novel biomolecules such as nanobodies. Taguchi method is a statistical experimental design to study the effect of multiple variables in biological processes. In an effort to overexpress a recombinant anti-human epidermal growth factor receptor type 2 (HER2) nanobody, we performed a detailed study to find optimal condition of temperature, induction, culture media, vector, and host strain, using Taguchi methodology. A total of 16 various experiments were designed. Total protein of the formulated cultures were assessed by Bradford test and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by size exclusion high performance liquid chromatography to quantify the relative concentration of the nanobody in different expression settings. Western blotting was performed to confirm the expression of the anti-HER2 nanobody. When, individually, optimum parameters determined by Taguchi were applied, including *SHuffle* strain cultured in LB medium, induced with 0.4 mM isopropyl-*β*-D-thio-galactoside for 18 h at 24°C, production yield further increased by about 9% (25.4 mg/L), compared to the highest expression setting. Flow cytometry and enzyme-linked immunosorbent assay result indicated improved protein binding in optimized conditions. Overall, our findings provide a basis for further investigations on economical production of recombinant nanobodies to improve production yield and activity.

KEYWORDS

HER2; nanobody; optimal condition; Taguchi methodology

Introduction

Overexpression of the membrane tyrosine kinase receptor human epidermal growth factor receptor type 2 (HER2) has long been known as a major prognostic factor in approximately 30% of breast cancers.[1,2] HER2 is a transmembrane protein from the tyrosine kinase receptor family. This family has four members which are collectively referred to as the HER1, HER2, HER3, and HER4.[3,4] HER2 plays an important physiological role in survival, differentiation, and cell growth regulation.[5] Several therapeutic agents targeting the HER2 signaling pathway have been introduced, among which anti-HER2 monoclonal antibodies (mAbs) appear more prosperous. The commercial monoclonal antibodies, trastuzumab and pertuzumab, specifically bind to different sites on the extracellular domain of HER2 to block its respective signaling and they have been effectively used to treat HER2-positive early stage and metastatic breast cancers.[6,7] Despite their rapid trust-gaining in diagnostic and therapeutic applications with more than 20 mAbs approved for clinical use, certain limitations including large size, immunogenicity, difficulty to penetrate dense tissues such as solid tumors and expensive production cost have hampered the extensive use of antibodies in such platforms.[8]

Naturally occurring in camelids, nanobodies are small (15 kDa) single-domain fragments possessing the full antigen-binding capacity of mAbs. Coding genes of nanobodies can be reversed transcribed from the mRNA of peripheral blood lymphocytes of animals immunized against a particular antigen. Further isolation of nanobody clones with high specificity is, generally, performed through phage display technology.[9] They are easily produced in microorganisms and exhibit very thermal stability, while their particular compact shape allows them to access cryptic epitopes, often inaccessible to conventional antibodies.[8,10]

In contrast to intact antibodies, nanobodies have been proved to be very efficiently produced in microbial hosts including *Escherichia coli*,[11] *Pichia pastoris*,[12] and *Saccharomyces cerevisiae*.[13] During the past two decades, *E. coli* has been utilized as the major workhorse for recombinant DNA technology to produce a variety of biopharmaceutical products.[14] Several strains of *E. coli* have been fully characterized at the molecular level and the introduction of foreign genes into them is accomplished through simple methods.[15] Considering two to threefold lower average cost-of-goods of microbial expression compared to mammalian expression, this is considered a key advantage of nanobodies.[10]

Due to administration of high doses of antibody over a long period of time, immunotherapies often impose large amounts of purified antibody per patient.[3] Hence, optimization of manufacturing processes is a critical step in the production of nanobodies as cost-effective therapeutic agents. Optimum

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